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## The Nucleus.

a report by  
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While, in recent years, cytologists have been able to ascribe some probable function and unity of form to most of the components of cells in different species, the nucleolus remains one of the least explicable problems of cytology today. This body is a conspicuous, typical part of the nucleus of most cells of higher animals. In different forms and situations, however, it reveals a diversity that is not equalled by other cell components.

As much as the nucleolus undergoes a typical cycle in mitosis, disappearing at the metaphase and reappearing at the very early anaphase, a link between the chromosomes and the nucleolus has long been suspected. The following papers are indicative of the early approach to the problem:

Zubile, 1928a, 1931; Filay, 1930; Montgomery, 1899; de Mol, 1927.

The work of Zubile, Filay and de Mol, is out-dated and superseded by more modern developments; Montgomery's is an exhaustive review, largely historical, of the nucleolus up to 1898. The author does not regard it as essential to discuss the historical aspect of nucleolar research. In that point, Sharp, 1934, Hofmeister, 1848, might be consulted. Gates (1937) reviews the more modern works.

Modern work on a number of species of animals and plants indicates a close connection between the nucleolus and a secondary constitution of a chromosome. Heitz (1931a + b) has shown that

the position and number of the nucleoli in various varieties of *Vicia* follow the position and number of the secondary constrictions of particular chromosomes in the ~~resting~~ <sup>reconstructing</sup> nucleus in telophase. More recently, McClintock (1934) has described the formation of the nucleolus at a chromatic region setting off a satellite - constriction in Zea mays PTC's. Furthermore, with the assistance of X-Rays, a reciprocal translocation was secured between the 6th and the 9th chromosomes, such that the break in chromosome 6 occurred through the ~~other~~ chromatic region. The number and position of the nucleoli in the products of subsequent crosses depended on the number <sup>and position</sup> of such regions, whence it is called the "nucleolus organizer". When no organizer is present, no single nucleolus is formed, but a number of "nucleolus-like" bodies appear to have formed by the coalescence of matrix along the length of the chromosomes. Also, when a nucleolus is present, the extent of chromosomal matrix is inversely proportional to the extent of the nucleolus. McClintock suggests, therefore, that the nucleolus has some close relationship to the chromosomal matrix. Venkatesai (1939) who confirms this process in many species of *Allium*, attempts to give a chemical basis for this relationship. At the present stage of our knowledge of the chemistry of nucleolus and matrix, any conclusions are very tentative indeed.

This relation between "constrictions" and nucleolus is confirmed for many other organisms (see, for example, Grasse + Tesperozz "38".) In prophase, presumably, nucleolus contributes to matrix. (?)

A few instances have been described where the mitotic history of the nucleolus apparently does not conform with the account given for *Zea*. It must be emphasized first of all that no satisfactory description of the prophase history of the nucleolus is available. It appears to fragment, dissolve, become extruded into the cytoplasm, and disappear. See, for instance, Francini ("38", a, b.), and Zirkle, 1928.

Frankel (1937), describes an 'aberrant' case in the meiosis in a few strains of certain species of *Fritillaria*. In *F. obliqua*, the nucleolus, instead of a spheroid more or less centrally located, is a cap on the periphery of the interphase reticulum, just inside the nuclear membrane. 3 chromosomes may be attached to it, presumably the 3 which show secondary constrictions in somatic metaphase. Before metaphase I, the nucleolus becomes globular and separates from the chromosomes. The appearance suggests the sudden removal of the pressure exerted by the nuclear membrane. This nucleolus persists, may fragment, and is distributed at random to the tetrad of spores. *F. plurifolia* exhibits somewhat similar behavior, but a smaller proportion of "cap nucleoli" are seen.

In other species, particularly *F. citrina*, the Telophase is peculiar. Globules appear at the ends of the chromosomes, looking like nucleoli, and separate as the figure polarizes. These disappear for Prophase II, reappear at Telophase II. After "osmotic" filtration, these globules, as well as the regular nucleoli, are positive to Feulgen-Nucleohalazur, indicating chemical similarity to chromosomes. There is no evidence of matrix coalescence as McClintock's theory demands.

In another genus, *Dryja sativum* (rice) Selvin ('30) also finds peculiar behavior in the meiosis of some strains. In early prophase, the nucleolus buds off a secondary nucleolus which remains attached. Different races vary in the details. Similar phenomena occur in megasporocyte divisions. Selvin regards (without great foundation) that the primary nucleolus contributes to the achromatic figure and the secondary to the substance of the chromosomes. In plants, the nucleoli appear to remain quiescent in the inter-mitotic phase, and do not show much specialization.

In animal material, there are also many peculiar specializations, but these occur in "resting" stages. There are three well-known instances where the nucleolus may contribute directly to the growth or secretion of cells. These are the developing

oocytes in many gastropods - e.g., *Limnaea* or *Patella*; in the spinning silk glands of many lepidopterans, and in the keratinizing layers of the dermal epithelium. However, these instances are by no means thoroughly established or generally accepted (Wilson, '25, p. 346, et al.).

Ford has worked on the egg development in *Lymnea*. Here, the typical "nucleoles of the early oogonium differentiates into a basophilic and an oxyphil set. The latter is extruded, and in conjunction with Golgi and chromosomes play some role in yolk-sphere formation. (Ford, '22.) At the end of oogenesis the basophil part fragments and distributes the throughout the cytoplasm. He considers there is a correlation between the size of the nucleoli and the degree of cell activity. Similar phenomena are noted in many other sorts of oocyte material. Wilson ('25) cites Ferguson on various tracheates where the chromosomes of the germinal vesicle enlarge, become less basophilic and assume a "lampbrush" form. The nucleoli then becomes basophilic, seeming to act as a reservoir of chromatin, although there is no direct chemical evidence for this. There are scattered references of uncertain significance, in the literature, reporting the reaction of similar nucleoli to Feulgen. This question is still open. In certain fish, the heterochromatin nucleoli split out into chromosome-like segments, and at one time were mistaken.

for such. Also, in some cases, the nucleolus persists after the breakdown of the germinal vesicle, and thus must contribute to the egg cytoplasm. This breakdown is the initiation of egg maturity; it is necessary before fertilization can ensue. The significance of the nucleoli here is speculative.

Nakashima (1917) is one of many authors who have examined nucleoli in silk glands. He concludes that silk (fibroin) is composed of extruded nucleoli. In these cells, the nucleus grows to a large size and branches so that a large surface is presented. There is a figure in Wilson, p. 86. At the same time, the nucleolus divides many times so that there are fragments throughout the nucleus. These particles seem to pass out of the ~~cell~~<sup>nuclear</sup> wall into the cytoplasm, where the staining reaction changes. The silk in the gland lumina has a similar reaction. The material worked on was *Pieris rapae* (a caterpillar) and *Neurotica postea* Wallen (the caddis-fly larva). The nature of this extension seems to demand further confirmation. Observations on live material would be most instructive.

Tredford (1924) has described the role of extruded nucleoli, Golgi and mitochondria in the formation of "keratohyalin" and keratin in the mouse epidermis. See also Cowdry (1928.)

This sort of work should demonstrate that we have not yet reached a unified conception of nucleolar function. In some instances

the nucleolus appears to have some role in secretions. It has been proposed that the chromosomes exert their genetic effects on cytoplasm through extruded nucleoli. But these, of course, are all speculations. Throughout any discussion of nucleolar fragmentation, one must keep in mind that the chromosomes in material inadequately prepared, may simulate nuclear products. As any case, extreme caution must be used in any nucleolar interpretation.

In most animals and plants, of course, the nucleolus has not been so closely studied. It is probable that their usual history is that outlined by Mc Clintock in relation to the Zea chromosomes.

Something is known of the physical situation of nucleoli. Macalpin, cited in Sedgwick '22, reported that they are active bodies, continually joining and fusing, in tissue-culture material. Chambers, 1924, on grasshopper (*Schistocerca carolina*), using - the micromanipulator, has found that the nucleolus can be readily moved in the cell, so that it is not firmly bound to any nuclear structure here. Tatesky (1938) gives a general account of ultra-centrifuge results, but see particularly Barnes, et al., '37.

After ultracentrifugation of *Helix aspersa*, and other species, spermatocytes, the nucleolus was found the heaviest object in the nuclei, with the chromosomes following. As in *Ollium sepa*,

the reference for which I cannot recall, the nucleoles may break through the nuclear membrane. Superficially, this is not in accordance with the supposed lipid nature of the nucleoles. (see infra) as lipoids are ordinarily less dense than water "protoplasm" incidentally, the mitochondria are the densest, the Golgi, <sup>and fat globules</sup> the lightest objects in cells. This density, implying ~~a~~ high concentration of its components is in accordance with the high stainability of the nucleoles even where a differential stain is not used (Lidberg, unpub.)

In the chemistry of the nucleoles, the most obscure mystery of nuclear biochemistry resides. Zirkle ('28, '29, '33a, b, c, d) reports variations in staining of the nucleoles by eosin hematoxylin after different fixatives. Underhill's data (1932) may be useful in interpreting the antagonistic effects of different fixatives in a mixture. These data concern the penetration of fixatives into pieces of guinea pig liver. The author's experience would confirm the list of relative penetrability given in Underhill's paper, except that his figure for formalin is far too low when applied to monoroot-tips.

The author, at another laboratory, has investigated, to some extent, the effect of fixatives, separately and in mixtures, on monoroot tips. (Lidberg, unpub.) This property would form the subject of another report. It has been found that acetic acid

so that they do not stain with Fe-Hem; formalin so that they do.  
~~not~~  
fixes nucleoli ~~to stain Fe Hem~~, formalin does <sup>of the two</sup> to mixtures, the  
acetic fixation (structurally) is evident, but some nucleoli in  
some nuclei stain. In some instances one nucleolus of two in  
a nucleus may stain, the other not, which proves nothing - yet,  
except that fixation produces erratic results.  
The subject is now being re-investigated. In connection with  
this, I am also studying the effects of various reagents on the  
apparent iso-electric points of the nucleolar proteins. As much  
as Bouin's fixation confuses <sup>apparent</sup> isoelectric point near 2.5 as the  
monocrotaline nucleoles, there probably is some ampholyte present, un-  
doubtedly a protein, or protein. This protein is not nucleoprotein,  
as it is negative to Feulgen (author's, unpubl.), as seems generally  
to be the case with typical nucleoli. The author believes that  
it is this IE P which determines the stainability of the nucleoli.  
The lower it is, the greater should be the stainability. This in-  
vestigation is now proceeding. The method essentially is Tolstovskoy's.

Other authors - Hensintai, 1939; Shiroki and Shigenaga,  
1933; Yasui, 1939; - claim the presence of lipid substances  
in nucleoli. As much as lipoids are universally present in  
protoplasm, we cannot yet claim an especial proportion in  
nucleoli; none of the investigators claim this. Indeed, Shiroki +  
Shigenaga find fats in "chromosomes, cytoplasm, nucleoli,  
nuclear ~~body~~ <sup>body</sup>, reticulum, and probably spindle fibers."  
There certainly is no basis, as Hensintai proposes - for

the formulation of the chemical cycle whereby chromosomal matrix, nucleoprotein is converted into nucleolus, lipid with a protein surface so it can be electrically charged.

Franzini, 1938, found in some orchids, finds a red coloration with Ruthenium chloride, interpreting this as a positive test for pectin or other polysaccharide. The author is continuing such histochemical tests in the Columbia histology laboratory. If, in Allium cepa, there is a protein in the nucleolus, as would seem, he hopes to find from specific amino acid tests, IEP changes after formalin and  $HNO_2$  treatment, and similar analytical methods, something of its nature and structure. It is inaccessible to analysis in the living cell because of its location and size. In all, this subject has remained one of the most obscure in cytology and biochemistry.

#### A brieviations of journals listed:

Ann Bot	Annals of Botany
B.A.	Biological Abstracts
JRMS	Journal of the Royal Microscopical Society
GJMS	Quarterly Journal of the Microscopical Society
J.M.	Journal of Morphology
St. Tech.	Stain Technology
Zeits. Zellf. und Anat.	Ztschrift für Zellforschung und Mikroskopisch Anatomie.

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1931 *Cytologia* 2: 85-105  
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